1994. Entry of the following amendments prior to examination is respectfully requested. The page and line numbers referred in the present document correspond to those of the application previously transmitted to the designated offices and to the published application, WO 95/09248.

AMENDMENTS

In the Specification:

At page 1, line 1, please delete "**METHODS AND COMPOSITIONS FOR EFFICIENT NUCLEIC ACID SEQUENCING.**"

A1 At page 1, line 2, after "The present application" please insert --is a nationalization of International Application No. PCT/US 94/10945, filed September 27, 1994; which--

A2 At page 1, line 6, please delete "co-pending".

In the Claims:

Please cancel claims ~~1-46~~ as may have been transmitted in the International Application, and substitute therefore new claims 1-48 as listed below:

~~1.~~ 49. A method for determining the sequence of a nucleic acid molecule, comprising the steps of:

(a) identifying sequences from the molecule by:

(i) hybridizing the molecule to complementary sequences of oligonucleotides from two sets of small oligonucleotide probes of known sequence, wherein the first set of probes are attached to a solid support and the second set of probes are labeled probes in solution; and

(ii) covalently bonding a hybridized oligonucleotide from said first set of probes to a hybridized oligonucleotide from said second set of probes;

(b) identifying overlapping stretches of sequence from the sequences identified in step (a); and

(c) assembling the nucleic acid sequence of the molecule from said overlapping sequences identified.

50 ~~2.~~ The method of claim ~~1~~ 49, wherein said hybridization is carried out in cycles.

51 ~~3.~~ A method for determining the sequence of a nucleic acid molecule, comprising the steps of:

(a) fragmenting the nucleic acid molecule to be sequenced to provide intermediate length nucleic acid fragments;

(b) identifying sequences from said fragments by:

(i) hybridizing the fragments to complementary sequences of oligonucleotides from two sets of small oligonucleotide probes of known sequence, wherein the first set of probes are attached to a solid support and the second set of probes are labeled probes in solution; and

(ii) covalently bonding a hybridized oligonucleotide from said first set of probes to a hybridized oligonucleotide from said second set of probes;

(c) identifying overlapping stretches of sequence from said sequences identified in step (b); and

(d) assembling the nucleic acid sequence of the molecule from said overlapping sequences identified.

52 4. The method of claim 51, wherein said fragments are sequentially hybridized to complementary sequences from two sets of small oligonucleotide probes of known sequence.

53 5. The method of claim 51, wherein said fragments are simultaneously hybridized to complementary sequences from two sets of small oligonucleotide probes of known sequence.

54 6. The method of claim 51, wherein said intermediate length nucleic acid fragments are between about 10 nucleotides and about 40 nucleotides in length and said small oligonucleotide probes are between about 4 nucleotides and about 9 nucleotides in length.

55 7. The method of claim 51, wherein said oligonucleotide probes hybridize to completely complementary sequences from said fragments.

56 8. The method of claim 51, wherein said oligonucleotide probes hybridize to immediately adjacent sequences from said fragments.

9. The method of claim 8, wherein said oligonucleotide probes hybridize to completely complementary and immediately adjacent sequences from said fragments.

10. The method of claim 3, wherein said oligonucleotide probes are covalently bonded by enzymatic ligation.

11. The method of claim 3, wherein said oligonucleotide probes are covalently bonded using a chemical ligating agent.

12. The method of claim 3, wherein step (b) comprises the steps of:

(a) contacting said first set of small attached oligonucleotide probes with said intermediate length nucleic acid fragments under hybridization conditions effective to allow only those fragments with a completely complementary sequence to hybridize to a probe, thereby forming primary complexes wherein the fragment has hybridized and free sequences;

(b) contacting said primary complexes with said second set of small labeled oligonucleotide probes under hybridization conditions effective to allow only those probes with completely complementary sequences to hybridize to a free fragment sequence, thereby forming secondary complexes wherein the fragment is hybridized to an attached probe and a labeled probe;

(c) covalently bonding said attached probed and said labeled probe;

(d) removing from said secondary complexes labeled probes that are not covalently bonded to an attached probe, thereby forming covalently bonded complexes;

(e) detecting said covalently bonded complexes by detecting the presence of the label; and

(f) identifying sequences from the nucleic acid fragments in said covalently bonded complexes by connecting the known sequences of the hybridized attached and labeled probes.

~~13.~~ 61 A method of nucleic acid sequencing comprising the steps of:

(a) fragmenting the nucleic acid to be sequenced to provide nucleic acid fragments of length T;

(b) preparing an array of immobilized oligonucleotide probes of known sequences and length F and a set of labeled oligonucleotide probes in solution of known sequences and length P, wherein F + P £ T; ← Page 13 of spec original claim Page 24 F+P ≤ T,

(c) contacting said array of immobilized oligonucleotide probes with said nucleic acid fragments under hybridization conditions effective to allow the formation of primary complexes with hybridized, completely complementary sequences of length F and non-hybridized fragment sequences of length T - F;

(d) contacting said complexes with said set of labeled oligonucleotide probes under hybridization conditions effective to allow only the formation of secondary complexes with hybridized, completely complementary sequences of length F and

immediately adjacent hybridized, completely complementary sequences of length P;

(e) covalently bonding said labeled oligonucleotide probes to said immediately adjacent immobilized oligonucleotide probes;

(f) detecting said secondary complexes by detecting the presence of the label;

(g) identifying sequences of length F + P from the nucleic acid fragments in said secondary complexes by combining the known sequences of the hybridized immobilized and labeled probes;

(h) determining stretches of said sequences of length F + P that overlap; and

(i) assembling the complete nucleic acid sequence from said overlapping sequences.

62 ~~14.~~ The method of claim 61 ~~13~~, wherein length T is about three times longer than length F.

63 ~~15.~~ The method of claim 61 ~~13~~, wherein length T is between about 10 nucleotides and about 40 nucleotides, length F is between about 4 nucleotides and about 9 nucleotides and length P is between about 4 nucleotides and about 9 nucleotides.

64 ~~16.~~ The method of claim 63 ~~15~~, wherein length T is about 20 nucleotides, length F is about 6 nucleotides and length P is between about 6 nucleotides.

112/2 support in spec. for definition

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17. The method of claim 13, wherein said immediately adjacent immobilized and labeled oligonucleotide probes are covalently bonded by enzymatic ligation.

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18. The method of claim 13, wherein said immediately adjacent immobilized and labeled oligonucleotide probes are covalently bonded using a chemical ligating agent.

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19. A method of nucleic acid sequencing comprising the steps of:

(a) fragmenting the nucleic acid to be sequenced to provide intermediate length nucleic acid fragments;

(b) contacting an array of immobilized small oligonucleotide probes of known sequences with said nucleic acid fragments under hybridization conditions effective to allow only those fragments with a completely complementary sequence to hybridize to a probe, thereby forming primary complexes wherein the fragment has hybridized and non-hybridized sequences;

(c) contacting said primary complexes with a set of labeled small oligonucleotide probes in solution of known sequences under hybridization conditions effective to allow only those probes with completely complementary sequences to hybridize to a non-hybridized fragment sequence, thereby forming secondary complexes wherein the fragment is hybridized to an immobilized probe and a labeled probe;

(d) covalently bonding said labeled oligonucleotide probes to said immediately adjacent immobilized oligonucleotide probes;

(e) removing from said secondary complexes labeled probes that are not covalently bonded to an immobilized probe, thereby forming covalently bonded complexes;

(f) detecting said covalently bonded complexes by detecting the presence of the label;

(g) identifying sequences from the nucleic acid fragments in said covalently bonded complexes by combining the known sequences of the hybridized immobilized and labeled probes;

(h) determining stretches of said sequences that overlap; and

(i) assembling the complete nucleic acid sequence from said overlapping sequences identified.

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20. The method of claim 19, wherein the nucleic acid is cloned DNA or chromosomal DNA.

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21. The method of claim 19, wherein the nucleic acid is mRNA.

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22. The method of claim 19, wherein the nucleic acid is fragmented by restriction enzyme digestion, ultrasound treatment, NaOH treatment or low pressure shearing.

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23. The method of claim 19, wherein the nucleic acid fragments are between about 10 nucleotides and about 100 nucleotides in length.

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24. The method of claim 19, wherein the oligonucleotide probes are between about 4 nucleotides and about 9 nucleotides in length.

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25. The method of claim 24, wherein the oligonucleotide probes are about 6 nucleotides in length.

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26. The method of claim 19, wherein said immobilized oligonucleotides are attached to a glass, polystyrene or teflon solid support.

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27. The method of claim 19, wherein said immobilized oligonucleotides are attached to a solid support via a phosphodiester linkage.

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28. The method of claim 19, wherein said immobilized oligonucleotides are attached to a solid support via a light-activated synthetic mechanism.

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29. The method of claim 19, wherein the labeled oligonucleotide probes are labeled with a non-radioactive isotope or a fluorescent dye.

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30. The method of claim 19, wherein the labeled oligonucleotide probes are labeled with ^{35}S, ^{32}P or ^{33}P.

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31. The method of claim 19, wherein said nucleic acid fragment or one of said oligonucleotide probes contains a modified base or a universal base.

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32. The method of claim 19, wherein labeled probes that are not covalently bonded to an immobilized probe are removed from the secondary complexes by stringent washing conditions.

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33. The method of claim 19, wherein said immediately adjacent probes are chemically bonded.

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34. The method of claim 19, wherein said immediately adjacent probes are ligated enzymatically.

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35. The method of claim 19, wherein multiple arrays of immobilized oligonucleotides are arranged in the form of a sequencing chip.

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36. A method of nucleic acid sequencing comprising the steps of:

(a) fragmenting the nucleic acid to be sequenced to provide nucleic acid fragments of between about 10 nucleotides and about 40 nucleotides in length;

(b) contacting an array of immobilized oligonucleotide probes with known sequences of between about 4 nucleotides and about 9 nucleotides in length with said nucleic

acid fragments under hybridization conditions effective to allow only those fragments with a completely complementary sequence to hybridize to a probe, thereby forming primary complexes wherein the fragment has hybridized and non-hybridized sequences;

(c) contacting said complexes with a set of ^{32}P-labeled or ^{33}P-labeled oligonucleotide probes with known sequences of between about 4 nucleotides and about 9 nucleotides in length under hybridization conditions effective to allow only those labeled probes with completely complementary sequences to hybridize to a non-hybridized fragment sequence, thereby forming secondary complexes wherein the fragment is hybridized to an immobilized probe and a ^{32}P-labeled or ^{33}P-labeled probe;

(d) ligating the immobilized probes and labeled probes that are immediately adjacent with a DNA ligase enzyme, thereby forming ligated secondary complexes;

(e) removing from the secondary complexes any non-ligated labeled probes;

(f) detecting said ligated secondary complexes by detecting the presence of the ^{32}P or ^{33}P label;

(g) identifying sequences from the nucleic acid fragments in said ligated secondary complexes by combining the known sequences of the ligated probes;

(h) determining stretches of said sequences that overlap; and

(i) assembling the complete nucleic acid sequence from said overlapping sequences.

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~~37.~~ A kit for use in nucleic acid sequencing, comprising a solid support chip having attached an arrangement of oligonucleotide probes of known sequences, said oligonucleotides being capable of taking part in hybridization reactions, a set of containers comprising solutions of labeled oligonucleotide probes of known sequences, and a ligating agent.

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~~38.~~ The kit of claim ~~37~~, wherein multiple chips of immobilized oligonucleotide probes are arranged in the form of a sequencing array.

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~~39.~~ The kit of claim ~~37~~, wherein the oligonucleotide probes are between about 4 nucleotides and about 9 nucleotides in length.

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~~40.~~ The kit of claim ~~39~~, wherein the oligonucleotide probes are about 6 nucleotides in length.

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~~41.~~ The kit of claim ~~37~~, wherein the oligonucleotide probes are attached to a glass, polystyrene or teflon solid support.

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~~42.~~ The kit of claim ~~37~~, wherein the oligonucleotide probes are attached to a solid support via a phosphodiester linkage.

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~~43.~~ The kit of claim ~~37~~, wherein the oligonucleotide probes are attached to a solid support via a light-activated synthetic mechanism.